SEQUENCE LISTING

<110> RIKEN

<120> Method for mutagenesis

<130> PH-1270US

<150> JP2000-237166

<170> PatentIn Ver. 2.0

<210> 1

<211> 720

<212> DNA

<213> Aequorea victoria

<220>

<221> CDS

<222> (1)..(717)

<400> 1

atg gtg agc aag ggc gag gag ctg ttc acc ggg gtg gtg ccc atc ctg 48 Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu 15 10 5 1

gtc gag ctg gac ggc gac gta aac ggc cac aag ttc agc gtg tcc ggc 96

Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly
20 25 30

gag ggc gag ggc gat gcc acc tac ggc aag ctg acc ctg aag ttc atc 144
Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe lle

35
40
45

tgc acc acc ggc aag ctg ccc gtg ccc tgg ccc acc ctc gtg acc acc 192

Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr

50 55 60

ctg acc tac ggc gtg cag tgc ttc agc cgc tac ccc gac cac atg aag 240
Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys
65 70 75 80

cag cac gac ttc ttc aag tcc gcc atg ccc gaa ggc tac gtc cag gag 288 Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu 85 90 95

cgc acc atc ttc ttc aag gac gac ggc aac tac aag acc cgc gcc gag 336
Arg Thr lle Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
100 105 110

gtg aag ttc gag ggc gac acc ctg gtg aac cgc atc gag ctg aag ggc 384 Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly 115 120 125

atc gac ttc aag gag gac ggc aac atc ctg ggg cac aag ctg gag tac 432

Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr

130 135 140

Fig.
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aac	tac	aac	agc	cac	aac	gtc	tat	atc	atg	gcc	gac	aag	cag	aag	aac	480
Asn	Tyr	Asn	Ser	His	Asn	Val	Tyr	lle	Met	Ala	Asp	Lys	Gln	Lys	Asn	
145					150					155					160	

ggc atc aag gtg aac ttc aag atc cgc cac aac atc gag gac ggc agc 528 Gly lle Lys Val Asn Phe Lys lle Arg His Asn lle Glu Asp Gly Ser 165 170 175

gtg cag ctc gcc gac cac tac cag cag aac acc ccc atc ggc gac ggc 576 Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly 180 185 190

ccc gtg ctg ctg ccc gac aac cac tac ctg agc acc cag tcc gcc ctg 624
Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu

195 200 205

agc aaa gac ccc aac gag aag cgc gat cac atg gtc ctg ctg gag ttc 672 Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe 210 215 220

gtg acc gcc gcg ggg atc act ctc ggc atg gac gag ctg tac aag taa 720 Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys 225

<210> 2

<211> 239

<212> PRT

<213> Aequorea victoria

<400> 2

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Met	Val	Ser	Lys	Gly	Glu	Glu	Leu	Phe	Thr	Gly	Val	Val	Pro	lle	Ļeu
1				5					10	-				15	

Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly
20 25 30

Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
35 40 45

Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
50 55 60

Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys
65 70 75 80

Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu 85 90 95

Arg Thr lle Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu 100 105 110

Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
115 120 125

Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
130
135
140

Asn Tyr Asn Ser His Asn Val Tyr lle Met Ala Asp Lys Gln Lys Asn 145 150 155 160

Gly lle Lys Val Asn Phe Lys lle Arg His Asn lle Glu Asp Gly Ser

165

170

175

Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
180 185 190

Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu 195 200 205

Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe 210 215 220

Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys 225 230 235

<210> 3

Dydriver Dauri

<211> 717

<212> DNA

<213> Aequorea victoria

<220>

<221> CDS

<222> (1)..(714)

<400> 3

atg agt aaa gga gaa gaa ctt ttc act gga gtt gtc cca att ctt gtt 48 Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val

gaa tta gat ggt gat gtt aat ggg cac aaa ttt tct gtc agt gga gag 96 Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu ggt gaa ggt gat gca aca tac gga aaa ctt acc ctt aaa ttt att tgc 144 Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys 35 40 45

act act gga aaa cta cct gtt cca tgg cca aca ctt gtc act act ttc 192

Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe

50 55 60

tct tat ggt gtt caa tgc ttt tca aga tac cca gat cat atg aaa cag 240 Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln 65 70 75 80

cat gac ttt ttc aag agt gcc atg ccc gaa ggt tat gta cag gaa aga 288 His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg 85 90 95

act ata ttt ttc aaa gat gac ggg aac tac aag aca cgt gct gaa gtc 336

Thr lle Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val

100 105 110

aag ttt gaa ggt gat acc ctt gtt aat aga atc gag tta aaa ggt att 384 Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile 115 120 125

gat ttt aaa gaa gat gga aac att ctt gga cac aaa ttg gaa tac aac 432 Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn 130 135 140

tat aac tca cac aat gta tac atc atg gca gac aaa caa aag aat gga 480

Tyr Asn Ser His Asn Val Tyr lle Met Ala Asp Lys Gln Lys Asn Gly
145 150 155 160

atc aaa gtt aac ttc aaa att aga cac aac att gaa gat gga agc gtt 528

Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val

165 170 175

caa cta gca gac cat tat caa caa aat act cca att ggc gat ggc cct 576 Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro lle Gly Asp Gly Pro 180 185 190

gtc ctt tta cca gac aac cat tac ctg tcc aca caa tct gcc ctt tcg 624

Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser

195 200 205

aaa gat ccc aac gaa aag aga cac atg gtc ctt ctt gag ttt gta 672 Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val 210 215 220

aca gct gct ggg att aca cat ggc atg gat gaa cta tac aaa taa 717

Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys

225 230 235

<210> 4

HUNNE

<211> 238

<212> PRT

<213> Aequorea victoria

<400> 4

Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val

5

10

15

Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu 20 25 30

Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe lle Cys
35 40 45

Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe
50 55 60

Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln 65 70 75 80

His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg 85 90 95

Thr lle Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val

Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile 115 120 125

Asp Phe Lys Glu Asp Gly Asn lle Leu Gly His Lys Leu Glu Tyr Asn 130 135 140

Tyr Asn Ser His Asn Val Tyr lle Met Ala Asp Lys Gln Lys Asn Gly
145 150 150 155 160

lle Lys Val Asn Phe Lys lle Arg His Asn Ile Glu Asp Gly Ser Val

Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro 180 185 190

Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser 195 200 205

Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val 210 215 220

Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys 225 230 235

<210> 5 <211> 27 <212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Primer

<400> 5

gcactgcacg ccccaggtca gggtggt

27

<210> 6

<211> 26

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Primer

<400> 6

gggcggactg gtagctcagg tagtgg

26

<210> 7

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<221> Degenerate

 $\langle 222 \rangle (9), (10)$ and (11)

 $\langle 223 \rangle$ "n" is a, t, c or g

<220>

<223> Description of Artificial Sequence:Primer

<400> 7

gcggactgnn ngctcaggta g

21

<210> 8

<211> 239

<212> PRT

<213> Aequorea victoria

<400> 8

Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro 11e Leu

l

Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly

Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe lle

Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr

Leu Thr Trp Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys

Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu

Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu

Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly

lle Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr

Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn

Gly lle Lys Val Asn Phe Lys lle Arg His Asn lle Glu Asp Gly Ser

Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Tyr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys <210> 9 <211> 239 <212> PRT <213> Aequorea victoria <400> 9 Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Arg Phe Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe lle

Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr

,	on The Tro Gly V	lal Gln Cvs Pl	ne Ser Arg Tyr Pr	o Asp His Met Lys	
I	ed III lib dia		75	80	
	65	70	13		
	Gln His Asp Phe	Phe Lys Ser A 85	la Met Pro Glu G	y Tyr Val Gln Glu 95	
	Arg Thr lle Phe	Phe Lys Asp A	Asp Gly Asn Tyr L 105	ys Thr Arg Ala Glu 110	
	Val Lys Phe Glu 115	Gly Asp Thr	Leu Val Asn Arg 1 120	le Glu Leu Lys Gly 125	
		Glu Asp Gly 135		His Lys Leu Glu Tyr 140	
	Asn Tyr Ile Se	r His Asn Val 150	Tyr lle Thr Ala	Asp Lys Gln Lys Asr 160	1)
	Gly lle Lys Al	a His Phe Lys 165	: lle Arg His Asn 170	Ile Glu Asp Gly Se	r
		la Asp His Ty 80	r Gln Gln Asn Thr 185	Pro Ile Gly Asp Gl	у
	Pro Val Leu L 195	eu Pro Asp As	n His Tyr Leu Se	r Tyr Gln Ser Ala Lo 205	eu
	Ser Lys Asp 1	oro Asn Glu L	ys Arg Asp His Me	t Val Leu Leu Glu P	'he

Val Thr Ala Ala Gly lle Thr Leu Gly Met Asp Glu Leu Tyr Lys 225 230 235